system lodges in the digestive tract, and the toxins produced there spread over the body." It is our intention to discuss this phase of botulism elsewhere in detail; nevertheless, the diagnostic significance should be emphasized. We had recently occasion to study, in cooperation with Dr. L. R. Vawter, of Reno, Nev., a cattle disease in which *B. botulinus* apparently exhibited invasive properties. Invariably the organism was isolated from the inflamed duodenum and jejunum, the liver, mesenteric lymph-nodes, etc.

It is noteworthy that our two attempts to isolate *B. botulinus* from the spleen were not successful. These results may, in part, be due to the fact that post-mortem invasion was made impossible by the early removal and careful preservation of the tissues after death.[1]

Summary.

B. botulinus, type B, has been isolated from the jejunal wall of a case of botulism fatal on the fifth day of the disease. Spleen cultures in two instances were negative for *B. botulinus*. Stool specimens of two clinical cases of botulism, obtained from two different outbreaks, contained *B. botulinus*, type A, on the sixth, seventh, and eleventh day, repectively, after the consumption of the causative meal. The methods of tissue and stool cultures are described. The importance of culturing the stools and tissues of all clinical cases of botulism is evident.

THE COMPARATIVE TOXICITY OF THYMOL AND CARVACROL (ISOTHYMOL.)[2]

By A. E. LIVINGSTON, Physiologist, United States Public Health Service.

Introduction.

Uncinariasis was shown by Stiles in 1903 to be quite prevalent in the southern portion of the United States, and his efforts are largely responsible for the fact that it is commonly diagnosed as such in this country at the present time. The treatment, which is now recognized as an important economic problem in many localities, usually consists of some vermifuge which will either kill or paralyze the parasite, causing it to release its hold on the intestinal wall and thus be swept from the digestive tract with the excreta. The ideal treatment should quickly kill all the parasites and at the same time produce no undesirable effects on the patient. Such a substance has not thus far been found. Among the various remedies which have been used may be mentioned

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[1] References:
(6) Proc. Soc. Exp. Biol. and Medicine, 1919, 17, p. 47.
(7) Arch. Int. Med., 1920, 26, p. 357.
(8) Jour. Am. Med. Assn., 1919, 73, p. 911.
(9) Boston Med. & Surg. Jour., 1920, July 29, 183, p. 139.

[2] From the Division of Pharmacology, Hygienic Laboratory, United States Public Health Service.

eucalyptus oil, naphthol, chloroform, male fern, calomel, thymol, oil of chenopodium, and chloroform in various combinations with other substances. Thymol has been used in hookworm disease for the past 40 years and in 1914 it constituted the principal remedy for this disease in this country. Our stock of thymol at that time was imported chiefly from Germany. This fact made our supply very uncertain and at times almost unobtainable, as pointed out by Motter (1914). With this decrease in supply, the price advanced to approximately five times that of previous years.

Because of these conditions, physicians began to prescribe oil of chenopodium (American wormseed oil), which had long been known to have an anthelmintic value. This oil is distilled in this country from *Chenopodium anthelminticum*, which makes our supply comparatively certain, but owing to its variability it is not established as a safe remedy for general use in hookworm disease.

Darling, Barber, and Hacker (1918), for instance, mention some of the objectionable features of thymol and oil of chenopodium. Among 123 cases which they treated with thymol, in doses ranging from 40 to 180 grains, the following effects were noted: Muscular incoordination, dizziness, inability to rise, marked burning in stomach, marked headache, vomiting, and albuminuria. Among 79 cases treated with oil of chenopodium, in doses varying from 0.75 to 3.0 c. c., the effects as compared with thymol were as follows: Dizziness more common, muscular incoordination more marked, inability to rise much more frequent, burning in stomach less marked, and headache less marked. Vomiting and albuminuria were also noticed, but the comparison with thymol was not definite. A semicomatose state was rarely noted. Five cases of deafness followed 3 c. c. doses; and in two cases death followed two treatments of 3 c. c., each administered with an interval of four days between. The critical observer may attribute many of these undesirable effects to the comparatively large doses used, but at best the literature regarding the treatment of hookworm disease indicates that new or additional specifics are desirable. Realizing this condition, Dr. Ralph W. McKee suggested that a study of carvacrol be made. This drug seemed to merit a preliminary investigation to determine its relative toxicity on animals before being tested clinically as a substitute for thymol in hookworm cases.

Carvacrol as a Possible Substitute for Thymol.

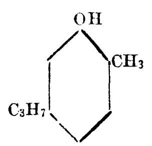
Sources of thymol and carvacrol.—Both thymol and carvacrol occur in nature and are found in several volatile oils. From *Thymus vulgaris*, a common plant indigenous to southern Europe, may be derived the "white oil of thyme." The less volatile and more valuable portion of this oil consists chiefly of thymol. Carvacrol is also present at times in this fraction, replacing part or all of the thymol.

Oils of origanum and savory contain carvacrol in varying concentrations, but none of these sources lends itself to production on a commercial scale. Samuel Clark Hood (1916), of the United States Department of Agriculture, has extended experiments over five years, showing that as much as 20 pounds of thymol per acre may be produced from horsemint, but that the cost of production at that time (1911–1915) was prohibitive as a commercial proposition.

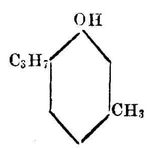
The thymol of commerce at the beginning of the war (1914) was derived almost entirely from ajowan seed which was grown in northern India and shipped to Europe, where the oil was extracted.

Carvacrol may be prepared artificially by a number of different methods as pointed out by Hixson (1918), but in most cases the materials used would result in a very expensive product. Hixson and McKee (1918) reported a new process for the manufacture of carvacrol on a large scale from spruce turpentine, which is obtained in large quantities as a by-product in the manufacture of wood pulp. This process seems to assure our supply for medical purposes, and probably at a much lower price than that commanded by thymol or oil of chenopodium.

Properties of thymol and carvacrol.—Carvacrol is an isomer of thymol, as seen by the following formulæ:



Carvacrol



Thymol

As it solidifies at 1° C. and boils at 236° C., it is encountered as a liquid; whereas thymol, having a melting point of 49.6° C. and a boiling point of 231.8° C., is a solid, even at body temperature. Carvacrol has a pungent aromatic taste, which much resembles that of thymol, and possesses a distinct local anesthetic property, as may be noticed when the drug is applied to the tongue. At the time of distillation it is a colorless oil, which, upon long standing, assumes a reddish brown color. It has a peculiar odor not unlike that of thymol, but much less pleasant.

Thymol, according to Seidell (1919), is soluble in water at 20° C. to the extent of about 880 parts per million. No definite statement in regard to the solubility of carvacrol in water has been found; but with the highest concentrations used in these experiments, which was 500 parts per million, a perfectly clear solution was obtained.

The United States Dispensatory (1918) quotes Martindale as stating that carvacrol is almost as actively germicidal as its isomer, thymol, but the data upon which this statement is based have not thus far been found by the writer. Hixson (1918) includes the state-

ment that "Recent comparative tests have shown carvacrol to be practically equal to and, in some cases, to possess greater antiseptic values than thymol," but this conclusion is based on only four viability tests on bacteria. Sollman (1919), the results of whose work were published after the present study was under way, concludes that carvacrol ranks with oil of chenopodium and thymol in toxicity for earthworms, that probably it is more irritant and toxic for dogs than thymol, and that it deserves a careful clinical trial as an anthelmintic substitute for thymol. The writer has been advised that clinicians have found that patients objected to its use on account of the taste. It is possible that a method of administration might be adopted which would obviate this objection.

The factors, then, which indicate that carvacrol may be used as a substitute for thymol are as follows: Its source of supply in this country is assured; the raw materials from which it may be made are inexpensive; it is a liquid instead of a solid, as thymol, at body temperature, which gives it a better chance of coming in contact with all parts of the intestinal wall; it has a distinct local anesthetic property which, combined with its anthelmintic action, indicates a high efficiency; and, being an isomer of thymol, its toxicity, as well as its anthelmintic properties, as shown by Sollman, is probably quite similar to that of thymol. In connection with the present toxicity experiments on rabbits and paramecia, it seemed that additional evidence in regard to its anthelmintic action, as indicated by tests on earthworms, might be valuable.

EXPERIMENTS ON RABBITS.

Dogs were found to be unsuitable for this work because they vomited soon after the drug was given, which, of course, made it possible that some of the drug might be lost. For this reason rabbits were chosen for further work. Both drugs have been administered by three methods: sometimes they were given in a 50 per cent solution in olive oil in gelatin capsules, sometimes in full strength in gelatin capsules, and at other times by means of a small inelastic catheter attached to an accurately graduated syringe. In the case of thymol the catheter method could be used only when the drug was dissolved in oil, while this method was applicable to carvacrol either in full strength or in oil. No comparison of results has been made to determine whether or not any difference in toxicity has resulted from these different methods of administration. The introduction by catheter and syringe, both being completely filled, has proved the most satisfactory from the standpoints of accuracy and convenience.

In all, 109 rabbits have been used, grouped, according to the drug used, into four series, namely, those receiving 50 per cent thymol dissolved in olive oil, those receiving thymol in the form of powder,

those receiving 50 per cent carvacrol in olive oil, and those receiving carvacrol in pure form. The dose for each ranged from 0.25 gram to 3 grams per kilo. A considerable individual variation in susceptibility was noticed, which was probably due, in part at least, to the variable amount of food in the stomach. The length of time of survival for each animal, or the time the animal was kept under observation, varied from a few hours to several weeks.

TABLE I.—*Results of different sized doses of thymol and carvacrol administered with and without olive oil.*

Thymol.						Carvacrol.					
In olive oil.			Not in oil.			In olive oil.			Not in oil.		
No. of animal.	Dose in gm. per kg.	Days survived.	No. of animal.	Dose in gm. per kg.	Days survived.	No. of animal.	Dose in gm. per kg.	Days survived.	No. of animal.	Dose in gm. per kg.	Days survived.
123......	0.25	65+				107.....	0.25	65+			
124......	.25	15				108.....	.25	65+			
125......	.25	65+				109.....	.25	65+			
126......	.25	65+				106.....	.25	65+			
127......	.25	65+				105.....	.25	65+	59......	0.33	25
45.......	.5	120+	37......	0.5	24	31......	.5	3	41......	.5	8
48.......	.5	18	36......	.5	24	33......	.5	3	40......	.5	9
47.......	.5	31	34......	.5	19	32......	.5	10	43......	.5	32
44.......	.5	34	38......	.5	18	29......	.5	40	39......	.5	35
46.......	.5	54	35......	.5	34	112.....	.5	65+	86B....	.5	10
131......	.5	65+	88......	.5	16	115.....	.5	65+			
128......	.5	65+				114.....	.5	65+			
129......	.5	65+				118.....	.5	65+			
130......	.5	65+				116.....	.5	65+			
132......	.5	65+									
134......	.75	15	89......	.75	9	119.....	.75	65+	102.....	.75	2
136......	.75	20				122.....	.75	65+			
133......	.75	65+				121.....	.75	4			
135......	.75	65+				120.....	.75	27			
137......	.75	65+									
10.......	1.0	76				36......	1.0	95	103.....	1.0	3
12.......	1.0	37				23......	1.0	120+	86E....	1.0	[1] 20
11.......	1.0	5				28......	1.0	3	92......	1.0	[1] 18
9........	1.0	6				24......	1.0	2			
8........	1.0	6				25......	1.0	2			
138......	1.0	65+									
14.......	1.5	120+				20......	1.5	16	93......	1.5	2
16.......	1.5	120+				21......	1.5	120+	86C....	1.5	[1] 20
17.......	1.5	64				18......	1.5	5			
15.......	1.5	6				22......	1.5	2			
13.......	1.5	7				19......	1.5	3			
6........	2.0	79	95......	2.0	5	2.......	2.0	2	104.....	2.0	[1] 18
144......	2.0	3	112.....	2.0	15	142.....	2.0	1	115.....	2.0	4
4........	2.0	2	106.....	2.0	19	1.......	2.0	2	114.....	2.0	3
143......	2.0	21	126.....	2.0	11	141.....	2.0	1	118.....	2.0	1
5........	2.0	4	131.....	2.0	1	3.......	2.0	[1] 18	149.....	2.0	3
145......	3.0	1	100.....	3.0	4	147.....	3.0	1	62......	3.2	3
146......	3.0	1	101.....	3.0	8	148.....	3.0	1	119.....	3.0	6

[1] Hours.

NOTE.—The + sign means that the rabbit was discarded on the day indicated and probably lived even longer.

It may be observed from Table I that, in doses of 0.25 gram per kilo, 5 received thymol and 5 carvacrol. Only 1 rabbit died during the period of observation, which extended over 65 days. This rabbit lived 15 days after receiving thymol, which probably means that neither the thymol nor carvacrol in this dose was responsible for any deaths.

The next higher dose was 0.5 gram per kilo, 30 rabbits receiving this amount. Among the 14 animals receiving this dosage of carvacrol, 2 died within 3 days, whereas the remaining 12 lived 8 days or longer (most of them 5 weeks) after receiving the drug. Of the 16 rabbits receiving 0.5 gram of thymol per kilo, none died in less than 16 days.

Among 11 rabbits which received 0.75 gram per kilo, 5 received the carvacrol and 6 the thymol. The table shows that of the 5 receiving carvacrol, 1 died the second and 1 the fourth day, whereas the other 3 lived 27 days or longer. Of the 6 receiving thymol, the first to die lived 9 days, and the next 15 days. The other 4 lived 20 days or longer.

Of the 14 animals receiving 1 gram per kilo, 8 received carvacrol and 6 thymol. Six of the 8 rabbits receiving carvacrol died within 3 days, and 3 of the 6 rabbits receiving thymol died within 6 days. All others lived beyond the time when the drug might probably have been the cause of death.

The dose of 1.5 grams of carvacrol per kilo was used on 7 and of thymol on 5 rabbits. Of the 7 receiving carvacrol, 5 were dead within 5 days, and of the 5 animals receiving thymol, 2 were dead in 7 days. The only other rabbit which may possibly have died from either carvacrol or thymol lived 16 days after receiving carvacrol.

A still larger dose of 2 grams per kilo was given to 20 rabbits, 10 receiving carvacrol and 10 thymol. All 10 receiving carvacrol died within 4 days, whereas 4 of the 10 animals given thymol were dead in the same time, 1 in 5 days, 4 in 11 to 21 days, and 1 certainly survived all effects, since it lived 79 days.

Eight animals received 3 grams per kilo. Four of these received carvacrol and 4 received thymol. All died within 8 days.

No statement has thus far been made as to the comparative results when either thymol or carvacrol was given with or without olive oil. This, however, is shown in Table I. There was no intention of dwelling particularly on this question, but, since both methods were used, attention may be called to the results obtained. In regard to thymol in doses of 0.5 gram per kilo, no animal died in less than 16 days, and the length of time of survival varied to such an extent that it seems improbable for thymol to have been the cause of death in any case. Of these 16 rabbits receiving 0.5 gram per kilo, 10 received the thymol in olive oil and 6 received it in powdered form in gelatin capsules. In doses of 2 grams per kilo, however, 3 of the 5 animals receiving thymol in olive oil died within 4 days, whereas the same dose without oil in 5 cases produced 2 deaths in 5 days, and 1 of these animals showed on post-mortem examination that death was probably due to pneumonia.

Two rabbits received 3 grams of thymol per kilo in olive oil and both died within 1 day. In contrast to these, of the 2 rabbits which

received 3 grams of thymol without olive oil, 1 lived 4 and the other 8 days. When carvacrol is given in doses less than 2 grams per kilo, the table would indicate that if any difference in toxicity is shown, it would seem to be more toxic without than with the olive oil. This, however, is not the case when given in doses of 2 and 3 grams per kilo. Definite conclusions as to whether or not carvacrol or thymol is more toxic when given with olive oil can not be reached without further experiments. There seems to be no reason why carvacrol, being already in liquid form at body temperature, should be more toxic with an oil. On the other hand, since thymol is a solid at body temperature, it might be expected to be more toxic in the presence of a solvent such as an oil. This was reported by Stiles (1902) to be the case in dogs when thymol was followed by castor oil. Schultz (1915), on the other hand, says: "It was found that oils in which thymol readily dissolves, if used as a solvent, greatly increased the dose necessary to kill." The question which concerned us more in this connection was the relative toxicity of thymol and carvacrol. It is evident from Table II that there is no striking difference in the toxicity of the two substances when introduced into the stomach of rabbits. A close examination of the whole series, however, apparently shows a slightly greater toxicity for carvacrol than for thymol. This difference is certainly not enough to discourage a clinical trial in cases where conditions can be carefully controlled. As a matter of precaution, the dose used at first should, of course, be much smaller than the relative toxicity on rabbits would indicate.

EXPERIMENTS ON EARTHWORMS.

There is no intention of concluding directly that if carvacrol is toxic for earthworms it is likewise toxic for hookworms in the intestinal tract of man. Thymol, however, is generally known to have such an action; and if thymol and carvacrol affect earthworms in the same way, then we have reason to believe that they may also act the same on hookworms. Two species have been used, namely, *Helodrilus calignosa* (common garden worm) and *Allobophora foetida* (commonly known as the dung worm). In only a few cases, however, were the former observed, and hence all conclusions herein mentioned will refer to the latter. The worms were brought into the laboratory in some of the earth in which they were found and were kept in a large evaporating dish covered with a piece of plate glass. This prevented their escape and also kept the earth from becoming too dry.

Earthworms were used by Straub (1902) for the determination of the relative toxicity of various substances. He used glass dishes of a size such as to allow 50 c. c. of the solution tested to fill the dish to a depth of about 3 mm., and states that in well water the worms behaved normally for days.

TABLE II.—*Toxicity of thymol on earthworms.*

	Time required to kill for indicated parts per million.									
Parts per million	500		400		300		200		100	
	H.	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*
	0	20	0	25	0	25	0	35	2	0
	0	20	0	25	0	25	0	35	2	0
	0	15	0	15	0	25	0	40	1	40
	0	15	0	15	0	30	0	40	1	40
	0	17	0	20	0	20	0	30	2	30
	0	17	0	20	0	30	0	45	1	30
	0	20	0	25	0	30	0	50	1	45
	0	20	0	25	0	30	0	50	1	45
					0	45			1	45
					0	45			1	45
									2	0
									1	15
									1	15
									1	15
									1	30
									1	15
									1	45
									1	15
									1	15
									1	10
									1	10
									1	45
									1	45
									1	15
									1	15
									3	0
									3	0
Average	0	18	0	21	0	31	0	41	1	41

	Time required to kill for indicated parts per million.																	
Parts per million	90		80		70		60		50		40		30		20		10	
	H.	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*
	2	0	2	30	3	30	3	30	3	30	6	0	24	0	25	30	24	0
	2	0	2	30	3	30	3	30	3	30	6	0	5	0	6	30	31	0
	3	30	3	30	4	0	4	30	4	0	5	30	28	0	29	0	24	0
	3	30	3	30	6	0	5	30	5	0	6	30	18	30	18	30	24	0
	2	0	3	30	2	30	4	0	4	0	28	0	18	30	18	30	24	0
	5	30	3	30	2	30	4	0	5	0	5	0	18	30				
	3	0	4	0	3	30	6	30	4	15	7	0	18	30				
	3	0	4	30	4	30	4	0	4	45	7	0						
	1	30	3	0	3	30	4	0	7	0	7	0						
	3	30	3	0	5	0	3	20	4	45	17	30						
	4	0	4	45			3	20			17	30						
	4	0	4	45			3	30										
							4	0										
Average	3	8	3	35	3	51	4	8	4	35	10	17	18	43	19	36	25	24

TABLE III.—*Toxicity of carvacrol on earthworms.*

Time required to kill for indicated parts per million.

Parts per million	500		400		300		200		100		90		80	
	H.	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*
	0	20	0	25	0	25	0	45	1	45	2	30	3	0
	0	20	0	25	0	30	0	45	1	45	2	30	3	0
	0	15	0	15	0	25	0	40	1	40	3	30	3	30
	0	15	0	15	0	30	0	40	1	40	3	30	3	30
	0	17	0	30	0	40	1	0	1	45	6	0	3	30
	0	17	0	30	0	40	1	0	2	25	6	0	7	30
	0	20	0	25	0	40	1	0	1	15	4	15	4	0
	0	20	0	25	0	40	1	0	2	0	4	15	4	0
					0	45			1	0	3	0	4	0
					0	45			1	15	3	0	4	0
									1	15	3	0	4	30
									1	45	3	0		
									1	10				
									1	10				
									1	30				
									1	45				
									1	45				
									2	30				
									2	30				
									3	45				
Average	0	18	0	24	0	36	0	51	1	47	3	43	4	3

Parts per million	70		60		50		40		30		20		10	
	H.	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*	*H.*	*m.*
	4	0	4	45	1	15	7	30	23	0	20	30	24	20
	4	0	4	45	2	10	6	30	6	30	18	30	31	0
	4	30	6	30	5	0	6	30	6	30	19	0	53	0
	4	30	6	30	5	0	6	30	18	30	19	0	23	30
	4	0	5	30	7	30	5	0	18	30	19	0	23	30
	4	45	5	30	4	0	7	0	18	30	19	0	18	30
	4	0	3	20	4	0	7	0			19	0		
	7	0	4	0	6	30	8	0						
	4	30			5	0	18	30						
	4	30			7	0	18	30						
					7	0	22	0						
					7	0								
					8	0								
Average	4	35	5	6	5	20	10	16	15	15	19	9	28	58

The method used by Sollman (1919) and later by Macht (1919) consists in placing five worms in 100 c. c. of the solution to be tested in a conical urine glass. This method was used in the first few of these experiments, but the control experiments carried out by immersing the worms in distilled water, tap water, and tap water containing some of the earth in which the worms live, showed that two worms in each glass did not remain in good condition over long periods of time. In these fluids, in some cases, the worms were found to be dead the next day. This suggested some cause other than the drug itself. Drowning was suspected, and petri dishes were substituted for the urine glasses. Fifty cubic centimeters of the control, or of the solution containing the drug to be tested, were placed in a petri dish 15 cm. in diameter, and the dish was kept closed. By using 50 c. c. in these dishes, the control fluids caused no effect, even though the worms were confined in them for more than a week. The solution is thus too shallow to complicate the results by a possibility of drowning, yet deep enough to insure an exposure of the worms to the drug at all times and to prevent an appreciable loss of the drug. All experiments have been made at room temperature, which ranges in the neighborhood of 21° C. Fresh solutions of the drugs in distilled water were always used. Before making an observation the worms were immersed in water to remove adhering particles of earth. Two were then placed in each dish and closely observed for a few minutes. Further observations were made at intervals of 15 minutes for several hours, or until death occurred.

The findings for thymol and carvacrol agree in every respect with the possible exception of the length of time required to kill. The response varies in intensity, but not in character, with the concentration of the solution used. In the strongest solution (500 parts per million) the worms at first make a few frantic efforts to escape. These are quickly followed by whipping and writhing movements, which rapidly become more and more feeble until all motion ceases, which occurs within from 10 to 15 minutes. Incidentally, it is of interest to note that within a few seconds after the worms are placed in either solution they begin to discharge a round mass of yellow substance from the mid-dorsal portion of each segment, which gives a beaded appearance along the dorsal surface. It is soon thrown off and gives the solution a yellow tint. No evidence has been found which would indicate that this is a waste product, thrown off on account of a stimulating action of the drug, or a protective mechanism designed to neutralize in some way the irritating action of substances with which they may come in contact. With the lower concentration these reactions require more time to develop, so that in a solution of 100 parts per million the movements continue for an average of about 90 minutes. Cessation of spontaneous movements is not

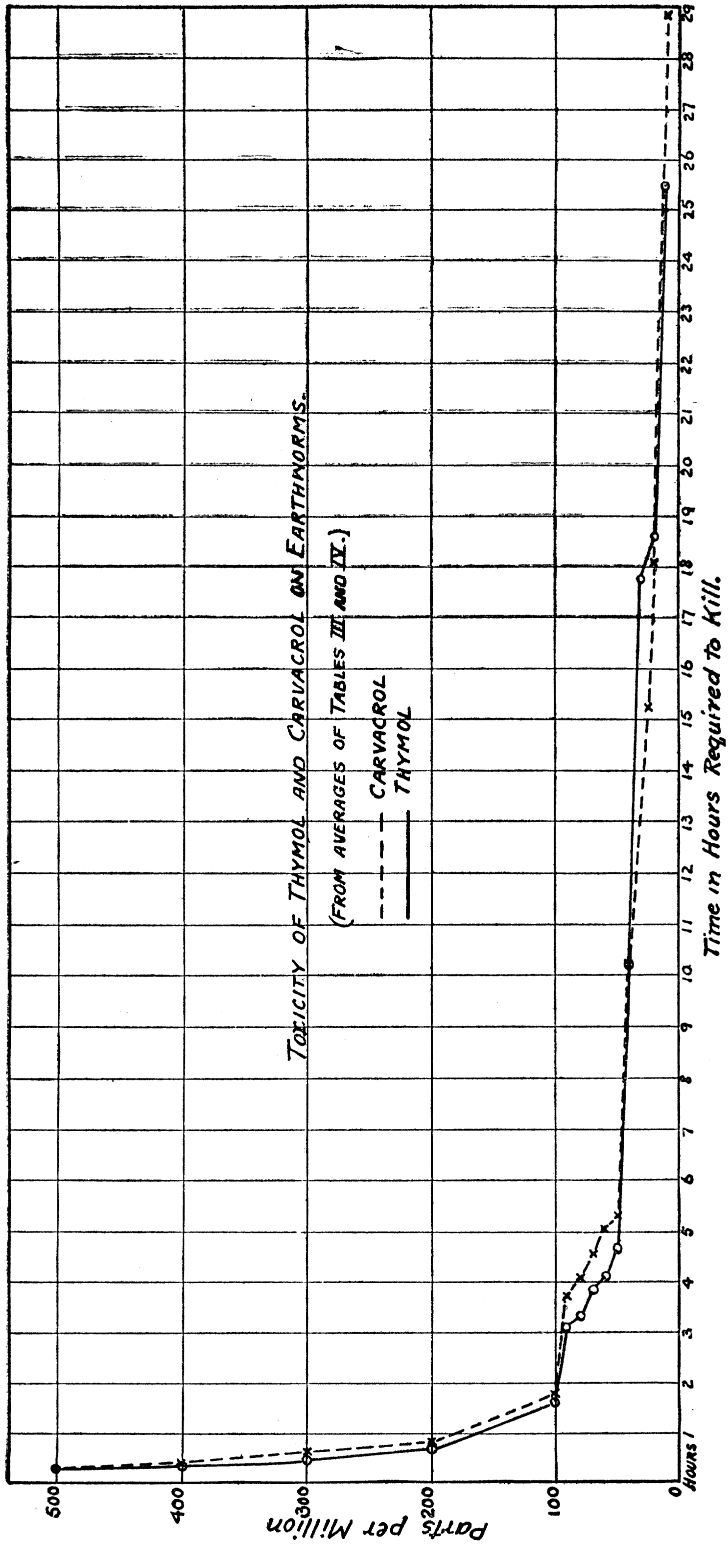

TOXICITY OF THYMOL AND CARVACROL ON EARTHWORMS.
(FROM AVERAGES OF TABLES III AND IV.)
CARVACROL
THYMOL
Time in Hours Required to Kill.
HOURS
Parts per Million
500
400
300
200
100
0

FIG. 1.

considered a criterion of the time of death, because movements may still be produced by mechanical stimulation for a somewhat longer time. The exact time at which no reaction can be obtained by mechanical stimulation has been considered as the time of death. The length of time required to produce death by the different concentrations is shown in Tables II and III. The corresponding curves (Fig. 1) plotted from these figures reveal the fact that with most concentrations carvacrol requires slightly more time than thymol in which to produce death. This difference is so slight, however, that it may possibly lie within the limits of experimental error. An irregularity in the curves for both thymol and carvacrol is shown for concentrations between 50 and 100 parts per million. No explanation can be given at present for this variation.

EXPERIMENTS WITH PARAMECIA.

For the purpose of supplementing the toxicity experiments with a unicellular type, the paramecium was selected. It was soon found by a few preliminary tests that this organism is rapidly killed by 10 parts of the drug per 100,000 parts of distilled water, whereas 1 part per 100,000 does not kill. The experiments were therefore confined between these limits of concentration. The method usually followed consisted in arranging nine small test tubes in a rack and placing in them, respectively, 1, 2, 3, 4, 5, 6, 7, 8, and 9 c. c. of a 0.01 per cent solution of the drug to be tested. In the same order was added 8, 7, 6, 5, 4, 3, 2, 1, and 0 c. c. of distilled water. Each tube, therefore, contained 9 c. c. To each tube was now added 1 c. c. of water containing the paramecia, which resulted in dilutions, mentioned in the same order, of 1, 2, 3, 4, 5, 6, 7, 8, and 9 parts per 100,000. The data card as used in tabulated form is shown below:

DATA CARD.

DRUG (*Thymol or Carvacrol*) DATE..............................

	Number of tube.								
	1	2	3	4	5	6	7	8	9
Cubic centimeters of 0.01 per cent (thymol or carvacrol).	1.0	2.0	3.0	4.0	5.0	6.0	7.0	8.0	9.0
Cubic centimeters of distilled H_2O	8.0	7.0	6.0	5.0	4.0	3.0	2.0	1.0	0.0
Cubic centimeters of H_2O containing paramecia	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Resulting dilutions	.00001	.00002	.00003	.00004	.00005	.00006	.00007	.00008	.00009
Time of day culture was added	11:17:0	11:17:0	11:18:0	11:18:0	11:19:0	11:57:0	11:53:0	11:51:30	11:50:0
Time of day all were found dead	......	......	......	......	......	1:0:0	11:54:30	11:52:0	11:50:30
Time required to kill	......	......	......	......	......	1:30:0	0:1:30	0:0:30	0:0:30

It is necessary, especially with the stronger solutions, that the time be very carefully observed when the culture is added and when all

paramecia are dead. As soon as the culture was added, the tube was inverted once or twice to insure immediate contact of the paramecia with the drug. This was usually accomplished in one or two seconds. A drop of the mixture was immediately placed on a slide under the microscope and carefully observed. Fresh drops were thus examined as rapidly as possible until all paramecia were found to be dead. This procedure was repeated for the various dilutions.

The length of time of survival for all dilutions is shown in Table IV. From these averages the corresponding curves (Fig. 2) were plotted. It may be observed that between 5 and 7 parts per 100,000 there is a sharp break in the curve. Only after a day or more were all found to be dead in solutions of less than 5 parts per 100,000, whereas with 7 parts per 100,000 all were usually dead in two minutes or less. Of course, in any given tube some individuals were dead some time before all were dead. It was found that a much more definite end point could be obtained by taking the time at which all were dead instead of when approximately all were dead.

The culture of paramecia contained the individuals in numbers such as to permit the dilution as described above, and then with a magnification of about 50 times there were usually 4 to 6 in the field of the microscope at any one time. Thus, an approximate idea may be gained as to the number of paramecia exposed.

The point of most interest at the present time is the fact that there is no striking difference in the toxicity of the two substances for paramecia.

The author wishes to express his thanks to Dr. James E. Benedict, of the Smithsonian Institution, for assistance in the identification of the earthworms used, and to Prof. Carl Voegtlin, of the Hygienic Laboratory, for helpful suggestions throughout the work.

TABLE IV.—*Toxicity of thymol and carvacrol on paramecia.*

TIME REQUIRED TO KILL FOR INDICATED PARTS PER 100,000.

	Thymol.						Carvacrol.					
Parts per 100,000	4	5	6	7	8	9	4	5	6	7	8	9
	H. m. s.	*H. m. s.*	*H. m. s.*	*H. m. s.*	*H. m. s.*	*H. m. s.*	*H. m. s.*	*H. m. s.*	*H. m. s.*	*H. m. s.*	*H. m. s.*	*H. m. s.*
	24 0 0	20 0 0	0 22 0	0 3 0	0 1 0	0 0 30	......	......	1 1 0	0 1 0	0 0 30	0 0 30
	......	......	0 33 0	0 2 0	0 0 50	0 0 30	......	......	0 33 0	0 1 0	0 0 30	0 0 30
	......	5 0 0	0 11 0	0 1 30	0 0 30	0 0 30	......	......	1 3 0	0 1 30	0 0 30	0 0 30
	20 0 0	18 0 0	0 11 30	0 1 0	0 0 30	0 0 30	......	......	0 37 0	0 1 0	0 0 30	0 0 30
	......	......	0 30 0	0 2 0	0 0 40	0 0 30	......	......	......	0 2 0	0 0 15	0 0 15
	......	12 30 0	0 15 30	0 1 0	0 0 44	0 0 25	......	18 0 0	0 52 30	0 2 0	0 1 30	0 0 20
	28 0 0	9 0 0	0 12 30	0 2 40	0 0 42	0 0 35	......	......	1 0 0	0 1 50	0 0 50	0 0 22
	......	......	......	......	......	......	......	18 0 0	0 38 35	0 1 0	0 0 30	0 0 30
Average	24 0 0	10 45 0	0 19 22	0 1 52	0 0 42	0 0 30	......	18 0 0	0 49 18	0 1 25	0 0 41	0 0 26

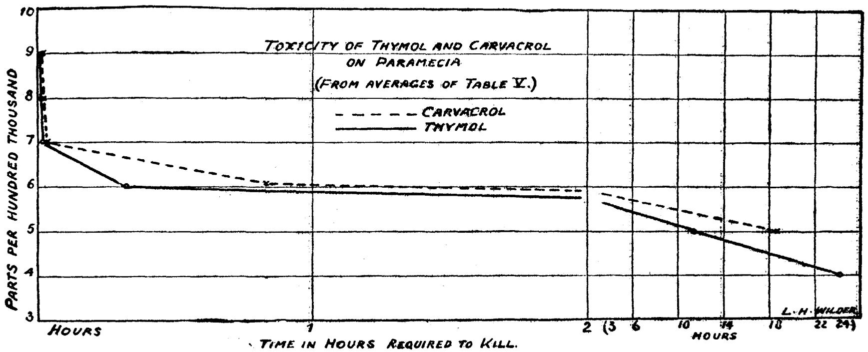


FIG. 2.

Conclusions.

1. The toxicity of thymol and of carvacrol on rabbits is essentially the same.
2. The toxicity of thymol and of carvacrol as tested on paramecia shows no striking difference.
3. Tests on earthworms indicate that the relative anthelmintic value of thymol and carvacrol is practically the same.

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RÉSUMÉ OF INSTRUCTIONS RELATING TO THE ENFORCEMENT OF THE UNITED STATES QUARANTINE REGULATIONS AT FOREIGN PORTS.

The following circular has recently been issued by Asst. Surg. Gen. Rupert Blue, in supervisory charge of medical inspection at European ports, stationed at Paris:

In order to secure uniformity of procedure at ports of embarkation, the following résumé of instructions is hereby issued for the information and guidance of all concerned: